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Amino Acid Composition of Developing Larval Freshwater Prawn *Macrobrachium rosenbergii*

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Abstract.—Amino acid profiles of freshwater prawn *Macrobrachium rosenbergii* were determined during early larval stages (I–IX) to provide some baseline biochemical information of the growing larvae. The larvae obtained from several females were stocked into three 250-L tanks at a density of 30 larvae/L. The feeding regime consisted of newly hatched *Artemia* nauplii and egg custard containing 5% cod liver oil. For each developmental stage, larval samples from each experimental tank were pooled, freeze dried and after acid hydrolysis, the quantification of amino acids was done by a reverse phase high performance liquid chromatography (N = 2). The highest represented of the total amino acids were for glutamic acid and phenylalanine (with cystine) with ranges from 13.4–16.6 and 9.7–11.5%, respectively, whereas tryptophan (1.4–1.6%), methionine (1.4–2.7%) and histidine (2.9–4.2%) were relatively lower. The levels of the following essential amino acids did not significantly change during larval development: arginine, leucine, phenylalanine (plus cystine), threonine, tryptophan and valine. Despite statistically significant changes that were observed in levels of some amino acids, amino acid composition appeared to be relatively unchanged during the larval growth. The results may suggest that the amino acid requirements of the freshwater prawn is relatively constant during larval life and can be satisfied by a suitable protein source that resembles the larval amino acid profile.

Freshwater prawn larval dependence on *Artemia* nauplii, which are very costly and nutritionally variable, is a major constraint in developing an aquaculture industry of freshwater prawn *Macrobrachium rosenbergii*. It is thus highly desirable to totally or partially replace *Artemia* with an effective and least-cost formulated diet. It is known that the single most important element of a viable diet is protein and the biological value of the dietary protein depends on its essential amino acid (EAA) composition (Shewbart et al. 1972; Van der Meer and Verdegem 1996). Studies have shown that crustaceans in general require the same 10 EAA required by most other animals (Cowey and Foster 1971; Miyajima et al. 1977). However, few investigations have been conducted on the quantitative requirements of EAA of crustacean species, particularly during their larval life.

It has been shown that the amino acid profile of the eggs and/or whole body tissue can be used in predicting the dietary amino acid requirement of the test animal (Benitez 1989; Penaflorida 1989; Das et al. 1996). Review of available literature, however, indicates that there have been no studies concerning amino acid profile of larval *M. rosenbergii*. Therefore, the present investigation was conducted to furnish some information on amino acid profile of larval *M. rosenbergii*. It is believed that such information would be useful in formulating suitable artificial diets for larval *M. rosenbergii*.

Materials and Methods

The experiment was conducted at the Hatchery and Pond Complex of Universiti Putra Malaysia. Circular fibreglass tanks (inner diameter: 90 cm; height: 60 cm) holding 250 L of brackish water were used for rearing *M. rosenbergii* larvae. The water was prepared by mixing aged, dechlorinated tap water and natural seawater to provide a salinity range of 10–12 ppt. The seawater was passed through a 1-μm filter prior to mixing with tap water. Tanks were provided with constant aeration and placed under transparent roofing and black plastic netting to allow a minimum of 10 h of indirect, non-intensive sunlight.

M. rosenbergii larvae were obtained from several wild-caught gravid females. The total number of the larvae was estimated volumetrically (8–16 samples using a 30-mL beaker) and were stocked into three tanks at a density of 30 larvae/L in a total water volume of 250 L. Larvae were reared with a "modified static green water" system (Cheah and Ang 1979; Ang and Cheah 1986) with the modification that the tanks were not seeded by green water at the beginning of culture period but rather the mixture of aged, dechlorinated tap water and natural seawater described earlier was used to develop the algal mat. The algal mat removes toxic wastes and by-products, such as ammonia and nitrite. While the algae acts as a filter, it does not provide any nutrition for larval *M. rosenbergii* either by direct consumption or indirectly through uptake by *Artemia* (Maddox and Manzi 1976).

Water quality parameters, including temperature, dissolved oxygen, pH, ammonia nitrogen, and salinity, were monitored 2 to 3 times weekly using a maximum-minimum thermometer, YSI oxygen meter model 57, Schott Gerate pH meter model CG 837, Lamotte Low Range Ammonia Kit and a hand-held Atago refractometer model 8808, respectively. The water quality parameters were relatively constant throughout the duration of the study. Water temperature varied from 25 to 29.3 C while dissolved oxygen and salinity were maintained above 6.5 mg/L and 12 ± 1 ppt, respectively. The pH ranged from 8.0 to 8.3 and total ammonia-nitrogen remained below 0.8 mg/L. The variations in water quality parameters monitored in this study were within the acceptable range for rearing *M. rosenbergii* larvae (Armstrong et al. 1978; Daniels et al. 1992). No specific stress was observed during the rearing period.

The most suitable and widely practiced feeding regime in *M. rosenbergii* larval rearing consists of diurnal feeding with prepared diets followed by the last evening feeding with live *Artemia* (New 1990). The feeding regime adapted for this study consisted of a combination of newly hatched *Artemia* nauplii as live overnight feed and egg custard containing 5% cod liver oil as artificial feed for diurnal feeding (Alam et al. 1995). *Artemia* nauplii were given based on daily inspection of the tanks, beginning with 5–6 organisms per larva at stage II and gradually increased to 60 organisms per larva by stage IX when the study was terminated. Egg custard particle size was maintained using 225 to 600-μm sieves. Live food was fed once a day at 1800 h, whereas the artificial diet was distributed into four rations per day fed at 0800, 1100, 1400 and 1700 h.

Samples of larvae (except stage I) were collected when 80% of each tank population had attained the desired mean developmental stage (MDS) (Lovett and Felder 1988). Sampling started at late morning when the guts were empty. On sampling day, no artificial feed was provided prior to sampling. Due to loss of synchrony in growth after stage V, VI and VII, and stage VIII and IX were combined, respectively. Larvae stage X and stage XI were excluded since they became more benthic, and sampling would have disrupted the algal mat. For amino acid analysis of larvae stage I, approximately 500 larvae were collected before larvae were distributed among the experimental tanks. The following number

of the larvae were collected from each tank for each stage: for stage II and III, 200; for stage IV and V, 100; for stage VI/VII and VIII/IX, 50. The density was estimated for each tank before sampling for amino acid analysis. After each sampling, an appropriate volume of water was removed to compensate for larval removal and thus not affect the density. For each developmental stage, samples of larvae were pooled from the replicate tanks and washed with minimum volume of distilled water to remove excess salt, freeze-dried and were kept at −80 C until further work. Two subsamples from the pooled larvae were analyzed for amino acid analysis.

The sample preparation for determination of the amino acid profile consisted of: 1) protein precipitation and defatting of approximately 20 mg freeze-dried larval sample (Teshima et al. 1986); 2) hydrolysis of the protein using 4N methane sulfonic acid containing 0.2% tryptamine as described by Simpson et al. (1976) with the following modification: about 2 mg of sample protein was hydrolysed in 200 µl of the acid at 115 C for 22 h; and 3) partial neutralisation with 3.5 N NaOH to pH 2.2 ± 0.1 and filtration through Whatman cellulose nitrate membrane filter (0.45-µm pore size). Amino acids were derivatized using o-phthaldialdehyde/2-mercaptoethanol reagent (Lee and Drescher 1978). The quantification of amino acids was done by a reverse phase high performance liquid chromatography system (Waters) equipped with a Bio-Rad prepared column (Bio-Sil ODS-5S,150 mm × 4 mm, Bio-Rad Laboratories, Hercules, California, USA) in duplicate. Methanol : tetrahydrofuran : 50 mM sodium acetate and 50 mM dibasic sodium phosphate pH 6.8 (2:2:96), and 65% methanol were used as solvents. Amino acids were identified from retention indices obtained by using an amino acid standard (Sigma Chemicals, St. Louis, Missouri, USA). Amino acid results were expressed as weight percentage of total amino acids. The essential amino acid ratio (A/E) was calculated as (essential amino acid content/total essential amino acid content including cystine) × 100 (Penaflorida 1989).

The weight percentage of total amino acids and the essential amino acid ratio (A/E) were arcsine-square root transformed (Steel and Torrie 1980) and tested for statistical significance ($P < 0.05$) by one-way analysis of variance and, where appropriate, by Tukey's HSD test to determine differences among the means using SPSS release 6.0 software (SPSS Inc., Chicago, Illinois, USA).

Results

Tables 1 and 2 represent the essential and nonessential amino acid profiles of the larval *M. rosenbergii* during various larval stages, respectively. Glutamic acid and phenylalanine (with cystine) were higher represented and ranged from 13.4–16.6 and 9.7–11.5%, respectively. Tryptophan (1.4–1.6%), methionine (1.4–2.7%) and histidine (2.9–4.2%) were relatively lower. The levels of following essential amino acids were not significantly changed during larval development: arginine, isoleucine, leucine, phenylalanine (plus cystine), threonine, tryptophan and valine (Table 1). Alanine, however, was the only nonessential amino acid that showed no significant differences within larval stages (Table 2). The means and ranges of individual amino acids during larval development are also presented to provide an overall view of the larval amino acid composition of *M. rosenbergii.*

Table 3 shows the essential amino acid ratios (A/E) of different stages of larval *M. rosenbergii.* In general, the differences observed in A/E ratios paralleled those found in the essential amino acid profiles. The ratios of arginine, leucine, phenylalanine (plus cystine), tryptophan and valine did not change significantly during development. For comparative purpose, the A/E ratios of larval *P. japonicus* reared in sea water (specific gravity, 1.026) (Teshima et al. 1986) and *P. monodon* (salinity not stated) (Penaflorida 1989) are included in Table 3.

TABLE 1. *Essential amino acid profile (% of total amino acids) of* M. rosenbergii *at different stages of development. Value for each developmental stage is the mean of two replicates. Average (± SD) and range of values for each amino acid during the larval life are presented. Means within the same row followed by different letters are significantly different according to Tukey's HSD test at 5% level of significance.*

Amino acids	Larval stages							Mean ± SD	Range
	I	II	III	IV	V	VI/VII	VIII/IX		
Arginine	8.9 a	7.9 a	7.2 a	7.1 a	7.3 a	6.9 a	7.0 a	7.5 ± 0.71	6.9–8.9
Histidine	3.4 ab	3.9 ab	2.9 a	3.3 ab	4.2 b	3.5 ab	3.2 ab	3.5 ± 0.44	2.9–4.2
Isoleucine	5.0 a	5.5 a	5.0 a	4.5 a	4.9 a	4.2 a	4.4 a	4.9 ± 0.45	4.2–5.5
Leucine	9.2 a	9.8 a	7.6 a	9.0 a	8.3 a	8.5 a	8.4 a	8.7 ± 0.71	7.6–9.8
Lysine	7.1 ab	8.9 b	6.4 a	7.0 ab	7.8 ab	8.0 ab	9.1 b	7.8 ± 1.00	6.4–9.1
Methionine	2.7 b	1.4 a	2.5 b	2.2 b	2.4 b	2.2 b	2.0 ab	2.2 ± 0.42	1.4–2.7
Phenylalanine[1]	10.6 a	10.8 a	11.1 a	11.5 a	10.5 a	10.2 a	9.7 a	10.6 ± 0.59	9.7–11.5
Threonine	4.7 a	4.6 a	4.8 a	4.6 a	4.9 a	4.9 a	4.5 a	4.7 ± 0.16	4.5–4.9
Tryptophan	1.6 a	1.5 a	1.6 a	1.4 a	1.5 a	1.5 a	1.4 a	1.5 ± 0.08	1.4–1.6
Valine	5.3 a	4.3 a	6.1 a	4.4 a	4.3 a	4.2 a	4.4 a	4.7 ± 0.72	4.2–6.1

[1] Phenylalanine plus cystine.

Discussion

The ontogenetic changes in amino acid content of the aquatic animals have not been examined extensively. Wilson and Poe (1985) reported no significant changes in amino acid profile of channel catfish ranging in weight from 30 to 863 g. The amino acid profile of whole *P. monodon* juvenile and adult muscle tissue did not differ significantly for most amino acids, whereas the zoea stage had significantly higher alanine, phenylalanine and tyrosine content but lower arginine, glutamic acid, methionine and tryptophan levels compared to juvenile and adult shrimp (Penaflorida 1989). Amino acid profile of muscle tissue of *P. monodon* from juvenile life to adult stage did not differ significantly for most of the amino acids (Liang et al. 1995). Despite the statistically significant differences that were observed in the levels of some amino acids through larval development of *M. rosenbergii,* the amino acid composition, especially essential amino acids, differed relatively little during the larval life as indicated by the narrow ranges and small standard deviations. Moreover, the statistical differences were mostly limited to a few stages. This may indicate that a suitable protein source would most likely fulfill the amino acid requirements of *M. rosenbergii* throughout the larval stages and possibly juvenile life as well since the amino acid profile of juvenile *M. rosenbergii* (Reed and D'Abramo 1989) is remarkably similar to the larval profile reported in this study.

TABLE 2. *Nonessential amino acid profile (% of total amino acids) of* M. rosenbergii *at different stages of development. Value for each developmental stage is the mean of two replicates. Average (± SD) and range of values for each amino acid during the larval life are presented. Means within the same row followed by different letters are significantly different according to Tukey's HSD test at 5% level of significance.*

Amino acids	Larval stages							Mean ± SD	Range
	I	II	III	IV	V	VI/VII	VIII/IX		
Alanine	5.5 a	5.9 a	5.7 a	6.2 a	6.2 a	6.3 a	5.8 a	5.9 ± 0.30	5.5–6.3
Aspartic acid	6.4 a	7.2 ab	8.9 b	8.3 b	7.5 ab	8.0 b	8.3 b	7.8 ± 0.83	6.4–8.9
Glutamic acid	13.4 a	14.8 ab	15.6 ab	16.3 b	14.3 ab	15.6 ab	16.6 b	15.2 ± 1.13	13.4–16.6
Glycine	7.4 b	3.2 a	4.8 ab	5.0 ab	6.9 b	6.5 b	6.3 b	5.7 ± 1.46	3.2–7.4
Serine	4.6 a	5.5 b	5.2 b	5.1 b	5.0 b	5.2 b	4.9 ab	5.1 ± 0.28	4.6–5.5
Tyrosine	4.3 ab	4.8 c	4.4 b	4.6 bc	4.5 bc	4.2 ab	4.0 a	4.4 ± 0.27	4.3–4.8

TABLE 3. *Essential amino acid (A/E) ratio[1] of* M. rosenbergii *at different stages of developments. The value for each developmental stage is mean of two replicates. Means in the same row followed by different letters are significantly different according to Tukey's HSD test at 5% level of significance. Data on larval* P. japonicus[2] *and* P. monodon[3] *are included for comparison.*

	Larval stages of *M. rosenbergii*						
Amino acids	I	II	III	IV	V	VI/VII	VIII/IX
Arginine	15.3 a	13.5 a	13.1 a	13.0 a	13.1 a	12.7 a	12.8 a
Histidine	5.7 ab	6.7 ab	5.2 a	6.1 ab	7.5 b	6.5 ab	5.9 ab
Isoleucine	8.5 ab	9.5 b	9.0 ab	8.2 ab	8.8 ab	7.8 a	8.2 ab
Leucine	15.7 a	16.7 a	13.8 a	16.3 a	14.8 a	15.7 a	15.6 a
Lysine	12.2 a	15.1 ab	11.7 a	12.7 ab	13.8 ab	14.9 ab	16.8 b
Methionine	4.6 b	2.4 a	4.6 b	3.9 b	4.3 b	4.1 b	3.7 b
Phenylalanine[4]	18.1 a	18.4 a	20.1 a	21.0 a	18.8 a	18.8 a	18.0 a
Threonine	8.0 a	7.8 a	8.8 bc	8.3 ab	8.7 bc	9.0 c	8.4 ab
Tryptophan	2.8 a	2.6 a	2.9 a	2.5 a	2.6 a	2.7 a	2.6 a
Valine	9.0 a	7.4 a	10.9 a	7.8 a	7.6 a	7.8 a	8.1 a

[1] (Essential amino acid/total essential amino acids including Cys) × 100.
[2] Teshima et al. (1986).
[3] Penaflorida (1989).
[4] Phenylalanine plus cystine.

In comparing amino acid values within or across species, the A/E ratio has been suggested to be a better index than amino acid content since it minimizes the effect of different sample preparations (Penaflorida 1989). The calculated A/E ratio of the whole body has also been shown to closely resemble the optimum amino acid balance needed by catfish (Wilson and Poe 1985) and is thought to provide good guidelines in estimating the dietary needs for fish for which requirements are not yet known (Ostrowski and Divakaran 1989). Some differences can be seen in comparing A/E ratio of *M. rosenbergii* larva with those reported for penaeid larvae. These differences in amino acid composition may indicate some structural and functional differences in protein content of different decapod larvae caused by various larval strategies such as brackish vs. marine environment. Furthermore, the metabolic and physiological needs for specific amino acids may also vary between species (Wilson and Poe 1985). However, based on the general similarity between quantitative results of this work with that reported for penaeid larvae (Teshima et al. 1986; Penaflorida 1989) in both A/E ratio and amino acid contents, it would appear that the protein composition of the larval freshwater prawn does not vary appreciably from those of the penaeid larvae. Furthermore, despite the limited validity of A/E ratio in comparing larval and juvenile *M. rosenbergii* (due to lack of cystine content in Reed and D'Abramo (1989) study), the overall resemblance may strengthen the notion that A/E ratio does not considerably change during the life cycle of freshwater prawn. Similar findings were reported for *P. monodon* (Penaflorida 1989).

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TABLE 3. *Extended.*

Larval stages of *M. rosenbergii*		*P. japonicus*	*P. monodon*
Mean (all stages)	Range		
13.4	12.7–15.3	15.6	15.0
5.4	5.2–7.5	4.7	5.0
8.6	7.8–9.5	10.1	9.0
15.5	13.8–16.3	13.4	15.8
13.9	11.7–16.8	14.4	17.4
3.9	2.4–4.6	6.3	5.3
19.0	18.0–21.0	12.1	11.9
8.4	7.8–9.0	6.5	8.4
2.7	2.5–2.9	7.0	1.8
8.4	7.4–10.9	9.7	10.9

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